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Prevalence of human papillomavirus type 16 and its variants in abnormal squamous cervical cells in Northeast Thailand

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Summary
Objectives: To investigate the prevalence of HPV, HPV16, and HPV16 variants in scraped cervical cells cytologically diagnosed as normal cervical cell and in formalin-fixed, paraffin-embedded tissues of cervical intraepithelial neoplasia II—III and squamous cervical carcinoma in Northeast Thailand.
Methods: All samples were subjected to PCR using consensus GP5+/GP6+ primers. HPV16 was genotyped by Southern blot hybridization and reverse line blot hybridization. The HPV16 E6 gene was amplified and sequenced.
Results: HPV infections were found in 33.8% of normal cervical cells, 97.3% of cervical intraepithelial neoplasia II—III, and 100% of squamous cervical carcinomas. The prevalence of HPV16 increased significantly with histological grade (normal cervical cell, 16.7%; cervical intraepithelial neoplasia II—III, 38.9%; squamous cervical carcinoma, 75%). The most common variant found was the Asian (As) (58.7%) followed by the European (E) lineage (41.3%). The HPV16 As lineages showed a risk association in 73.9% of squamous cervical cancer and 57.1% of cervical intraepithelial neoplasia II—III, while no increased risk was observed in the E lineages.
Conclusion: Our study demonstrates that HPV16, in particular the As variant, was the major causative agent associated with cervical cancer in Northeast Thailand, and our study suggests that some mutations of the E6 gene in this variant, which leads to amino acid changes, may be more carcinogenic.

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Introduction

The human papillomavirus (HPV) is a small, non-enveloped virus with double-stranded circular DNA. Anogenital HPV infection is usually a sexually transmitted disease. The viruses are divided into low-risk (LR) and high-risk (HR) groups depending on their oncogenic potential. HR HPVs (types 16, 18, 31, 33, 35, 45, 51, 52, 58, 59, 68, 73, and 82) are considered the main risk factor for the development of cervical cancer.[1] In Thai women, HR HPV has been detected in 57–100% of high-grade squamous intraepithelial lesions (HSILs) and squamous cell carcinomas, and in 27.3% of cases with low-grade squamous intraepithelial lesions (LSIL) and 3–20% of those with normal cytology.[2–6] The most common HPV type in squamous cell carcinoma has been shown to be HPV16 (38.4–65%) followed by HPV18, 58, 52, and 31.[2,3,7–9] In a recent study of women in Northeast Thailand, HPV16 was the type found most often in normal and low-grade lesions (ASCUS (atypical cells of undetermined significance)/LSIL) followed by HPV58.[10] Women with HPV16 infection have an estimated odds ratio of approximately 12.3 for cancer development compared to women without HPV.[4]

Different types of HPV are defined as having >10% variation in specified regions of the viral genome. Viruses varying from each other by 2–10% are referred to as subtypes and are infrequently observed, while those differing by <2% are seen more frequently and referred to as intratypic variants. HPV16 has been extensively sequenced to characterize variants[11] and is comprised of six major groups of variants. The HPV16 prototype belongs to the European (E) group but other variants include Asian (As), Asian American (AA), African 1 (Af1), African 2 (Af2), and North American 1 (NA1).[11] Several studies have now documented an association between HPV16 variants and the development of cervical cancer. In previous reports, non-European variants were found to be associated with an excess risk of cervical cancer. The HPV16 As variant was found to be prominent in Southeast Asia; thus, the association of this variant with cervical disease needs clarification.

The HPV16 protein sequence variations that correspond with nucleic acid variations may also affect the complex characteristics and change biological properties, such as immortalizing activity, transformation activity, and inactivation of tumor suppressor proteins, p53 and pRb. Host immunologic recognition may also change, perhaps in association with specific HLA haplotypes.[12] Several investigations have suggested an association of particular HPV molecular variants and the risk of cervical neoplasia.[12–17]

The aims of this study were: (1) to investigate the prevalence of HPV, HPV16, and HPV16 intratypic variation (variants) in scraped normal cervical cells and in formalin-fixed, paraffin-embedded tissues of abnormal squamous cervical cells from women in Northeast Thailand, by PCR using primers GP5+/GP6+, and (2) to evaluate the association between HPV16 infection and variant types with cervical disease status.

Materials and methods

Specimens

Cervical cells were obtained from women participating in routine cervical cancer screening. They were scraped with a wooden spatula and suspended in 5 mL of cold phosphate-buffered saline (PBS), pH 7.5. The suspended cells were washed twice with PBS and stored at −80 °C until used. Cytologically normal cervical cells ($N = 160$) diagnosed by Pap test were selected for DNA extraction. The formalin-fixed, paraffin-embedded cervical tissues were obtained from women undergoing biopsy or surgery at the Obstetrics and Gynecology Unit; these were histologically diagnosed as cervical intraepithelial neoplasia II–III ($N = 37$) and squamous cell carcinoma ($N = 40$). Six 5-μm-thick sections were cut from each paraffin block for DNA extraction. The DNA was extracted using a Puregene™ DNA purification system (Gentra, USA) following the manufacturer's instructions. All specimens were collected from Srinagarind Hospital patients who gave informed consent. This study was approved by the Khon Kaen University Ethics Committee for Human Research, as per the Helsinki Declaration.

HPV detection by PCR and nested PCR

The extracted DNA was qualified by β-globin gene detection using primers PC04 and GH20 (Table 1).[31] All qualified samples were firstly subjected to PCR amplification of the L1 region of HPV16 with GP5+/GP6+ consensus primers (Table 1).[32] The PCR was carried out in 50 μL, containing 200 ng of DNA, 0.2 mmol each dNTP, 3.5 mmol $MgCl_2$, 1 U of Taq DNA polymerase, and 50 pmol of the GP5+/GP6+ primers. The initial denaturation step at 94 °C for 4 min was followed by 40 cycles of a denaturation step at 94 °C for 1 min, an

Table 1 Primer sequences

Primer	Sequence (5′–3′)	Size (bp)	Reference
β-globin			
PC04	CAACTTCATCCACGTTCACC	268	31
GH20	GAAGAGCCAAGGACAGGTA		
HPV			
GP5+	TTTGTTACTGTGGTAGATACTAC	142	32
GP6+	GAAAAATAAACTGTAAATCATATTC		
HPV16-E6			
E6-F	TTGAACCGAAACCGGTTAGT	211	19
E6-R	GCATAAATCCCGAAAAGCAA		

annealing step at 40 °C for 2 min, and an elongation step at 72 °C for 1.5 min. The final elongation step was prolonged at 72 °C for 4 min. The final amplified product was 142 bp.

Samples that failed to produce detectable PCR products were re-amplified using the same primers in a nested PCR format. The number of cycles in the second round of PCR was decreased to 30, and 2.5 μL aliquots of the first round PCR was used as the template in the second round amplification.

HPV genotyping

The 142 bp PCR products of all HPV DNA-positive samples were subsequently genotyped for HPV16 by Southern blot hybridization technique using a specific biotin-labeled probe of HPV16 for screening. The specific biotin-labeled HPV16 probe was prepared by PCR using GP5+ and biotin-labeled GP6+ as primers, which target the L1 gene of HPV16 and plasmid with inserted HPV16 prototype as the template. The HPV16-positive samples were confirmed by home-brew reverse line blot (RLB) hybridization using an HPV16-specific oligonucleotide probe as described by van den Brule et al.[18] The biotin-labeled PCR product was amplified from extracted DNA of samples using GP5+ and biotin-labeled GP6+ as primers. Each amplified biotin-labeled PCR product was hybridized to the HPV16-specific oligonucleotide probe immobilized on a membrane.

Determination of HPV16 variants

The E6 gene (nt 46—256) of HPV16 was selected for the classification of HPV16 variants. The amplification of E6 gene mixture contained 3 mmol $MgCl_2$, 2 U Taq DNA polymerase, 0.2 mmol dNTP, 10 μM of each E6 primer (Table 1), and 200 ng of DNA template. Amplifications were started with an initial denaturation step at 95 °C for 5 min, followed by 40 cycles of denaturation step at 95 °C for 30 s, an annealing step at 55 °C for 45 s, and an elongation step at 72 °C for 1 min. A final extension step was performed at 72 °C for 7 min.[19] The PCR products were purified using the BILATEST PCR cleanup kit and subjected to automated sequencing at the Molecular Informatic Laboratory, Hong Kong.

The obtained nucleotide sequences of the E6 region at nt 46—256 were aligned and compared with those of the European prototype HPV sequence (HPV-16R) (GenBank accession No. **K02718**) types, available through the GenBank database (NCBI, National Institute of health, Bethesda, MD, USA), using the BLAST 2.0 software server (http://www.ncbi.nih.gov/BLAST/), BioEdit, and the Mutalin program. The HPV16 variants were classified into phylogenetic classes and subclasses on the basis of their sequence variation. The prototype sequence (HPV-16R) belonging to the European lineage was used for comparison and nucleotide position numbering. The E6 sequence patterns used for identification of phylogenetic classes were modified from Yamada et al.[11] as follows:

European (E): complete homology nucleotide deviated from HPV-16R;
Asian (As): ~178 nt: T→G;
African (Af1): ~132 nt: G→C, 143 nt: C→G, 145 nt: G→T;
African (Af2): ~109 nt: T→C, 132 nt: G→T, 143 nt: C→G, 145 nt: G→T;
North American (NA1): ~145 nt: G→T; and,
Asian American (AA): ~145 nt: G→T.

Statistical analysis

The association between HPV and cytologic or histologic category was analyzed by Chi-square test using 95% confidence intervals (CI).

Results

All of the samples ($N = 237$) were β-globin gene-positive indicating the presence of adequate cells in the samples. The HPV signal was detected at first round PCR in all HPV-positive paraffin-embedded tissue samples, whereas most of the HPV-positive scraped cervical cell samples showed the signal in the nested PCR. HPV DNAs were found in 33.8% (54/160) of normal cervical cells, 97.3% (36/37) of cervical intraepithelial neoplasia II—III, and 100% (40/40) of squamous cell carcinomas. The HPV infection rate increased significantly with the degree of disease status (OR 149.185; 95% CI 20.193—1102.168; $p = 0.000$) (Table 2).

For screening for HPV16 infection, the HPV-positive samples were genotyped by Southern blot hybridization assay and subsequently re-confirmed for HPV16 by RLB hybridization using an HPV16-specific oligonucleotide probe. Fifty-three subjects were positive for HPV16, which showed a significant trend of increase with the severity of the neoplasia ($p = 0.00$). HPV16 was present in 16.7% (9/54) of normal cervical cells, 38.9% (14/36) of cervical intraepithelial neoplasia II—III, and 75% (30/40) of squamous cell carcinomas, corresponding to an associated risk (OR 22.370; 95% CI 9.952—50.283) compared to normal cervical cells with HPV16 infection (Table 2). This result demonstrates the association of HPV infection with severe tissue abnormality. HPV16 was significantly associated with abnormal tissue and squamous cell carcinoma.

Table 2 Prevalence of HPV and HPV16 in normal scraped cervical cells and in paraffin-embedded tissue samples from patients with CIN II—III and SCC

Samples	NCC ($N = 160$)	CIN II—III ($N = 37$)	SCC ($N = 40$)	p-Value	OR	95% CI
HPV-positive	33.8% (54/160)	97.3% (36/37)	100% (40/40)	0.000	149.185	20.193—1102.168
HPV16-positive	16.7% (9/54)	38.9% (14/36)	75% (30/40)	0.000	22.370	9.952—50.283

HPV, human papillomavirus; OR, odds ratio; CI, confidence interval; NCC, normal cervical cell; CIN II—III, cervical intraepithelial neoplasia grade II—III; SCC, squamous cell carcinoma.

Table 3 Nucleotide sequence variation of the partial E6 gene (nt 46–256)

Ref. HPV-16R	Nucleotide of E6 gene																					Class	Predicted amino acid substitution
	103 A	108 T	109 T	112 G	114 A	131 A	132 G	134 A	135 A	136 G	137 T	138 T	143 G	145 G	161 C	176 G	178 T	183 T	188 G	246 G			
Specimen No.																							
NCC																							
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E-P	
57	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E-P	
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E-P	
24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E-P	
37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E-P	
48	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E-P	
49	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E-3685C	
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	As	D25E
108	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	As-647G	D25E
CIN II–III																							
2–6	-	-	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E-P	
2–11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E-P	
2–15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E-P	
2–16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	As	D25E
2–17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E-P	
2–19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	As	D25E
2–25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	As	D25E
2–27	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	G	-	-	-	-	As-132C	10T, D25E
2–36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E-P	
2–37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	As	D25E
2–39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	As	D25E
2–41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	As	D25E
2–42	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E-108A	F2Y
2–44	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	As	D25E
SCC																							
1–3	-	-	-	a	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	As-3684A	D25E
1–8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	As-647G	D25E
1–11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	As	D25E
1–13	-	-	-	a	-	-	-	-	-	-	-	-			-	-	G	-	-	-	-	As-647G	D25E
1–14	-	-	-	a	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	As-647G	D25E
1–16	-	-	-	a	-	-	-	-	-	-	-	-	-	-	-	A	G	-	-	-	-	As-176A	D25H
1–17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	As	D25E
1–19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	As	D25E

Table 3 (*Continued*)

Ref. HPV-16R	Nucleotide of E6 gene																				Class	Predicted amino acid substitution
	103 A	108 T	109 T	112 G	114 A	131 A	132 G	134 A	135 A	136 G	137 T	138 T	143 G	145 G	161 C	176 G	178 T	183 T	188 G	246 G		
1—21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	As-647G	D25E
1—26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	As	D25E
1—30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	As-647G	D25E
1—31	-	-	-	a	-	-	-	-	T	-	-	-	-	-	-	-	G	-	-	-	As-135T	K11N, D25E
1—32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	As	D25E
1—34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	As-647G	D25E
1—35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	As-647G	D25E
1—37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E-P	
1—38	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	E-145T	Q14D
1—40	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	E-145T	Q14D
1—42	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	As	D25E
1—44	-	-	-	C	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E-112C	Q3H, D4A
1—45	c	-	-	-	-	-	-	T	C	-	-	-	T	-	-	-	-	-	-	-	E-134T	K11Y, Q14H
1—46	-	-	-	-	-	-	-	-	-	-	G	C	-	-		-	G	-	-	-	As-137G	L12A, D25E
1—47	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	E-246	R48Q

HPV, human papillomavirus; HPV-16R, reference HPV16; NCC, normal cervical cell; CIN II—III, cervical intraepithelial neoplasia grade II—III; SCC, squamous cell carcinoma.
Phylogenetic classes are noted as E for European, As for Asian. 'P' following E refers to prototype. For subclasses, the number and the letter represent the nucleotide change at that position. At the top, nucleotide positions where variations were detected. In the columns, predicted substitution, the letter preceding the amino acid position refers to the reference HPV16 and the letter after refers to the substitution. The upper case; nucleotide and amino acid were changed, the lower case; nucleotide was changed but amino acid not changed.

Table 4 Distribution of HPV16 variants in normal and abnormal cervical squamous cells

HPV16 variants	Total	Cervical disease severity			*p*-Value	OR	95% CI
		NCC (%)	CIN II–III (%)	SCC (%)			
E lineage	19	7 (77.8)	6 (42.9)	6 (26.1)	0.022	0.137	0.025–0.763
As lineage	27	2 (22.2)	8 (57.1)	17 (73.9)	0.022	7.292	1.311–40.542
Total	46	9	14	23			

HPV, human papillomavirus; OR, odds ratio; CI, confidence interval; NCC, normal cervical cell; CIN II–III, cervical intraepithelial neoplasia grade II–III; SCC, squamous cell carcinoma.

HPV16 variants

Subjects with normal cervical cells (nine cases), cervical intraepithelial neoplasia II–III (14 cases), and squamous cell carcinomas (23 cases) who were HPV16-positive were further investigated for intratypic variation. The amplification product of the E6 gene at nt 46–256 was used for classification of HPV16 variants through a comparison with the modified data of Yamada et al.[11] as described above. All identified variants are listed in Table 3.

Among the HPV16-positive subjects, the most common variant was the HPV16 As lineage (58.7%) found in two normal cervical cells (22.2%), eight cervical intraepithelial neoplasia II–III (57.1%), and 17 squamous cell carcinomas (73.9%). Nineteen (41.3%) HPV16 E lineages presented with seven in normal cervical cells (77.8%), six in cervical intraepithelial neoplasia II–III (42.9%), and six in squamous cell carcinomas (26.1%) (Table 4). Comparison using 2×2 tables with the Fisher's exact test of the HPV16 As lineage frequency observed in normal cervical cells and those with cell abnormalities (cervical intraepithelial neoplasia II–III and squamous cell carcinomas), demonstrated that the HPV16 As lineage was significantly associated with the severity of cell abnormality (OR 7.292; 95% CI 1.311–40.542; $p = 0.022$).

Discussion

In the present study, we investigated the prevalence of HPV and HPV16 infection in women with a normal cytological diagnosis and those with a histological diagnosis of cervical intraepithelial neoplasia II–III and squamous cell carcinoma in Northeast Thailand. Using single PCR and nested GP5+/GP6+ PCR, the HPV DNA was detected in 33.8% of the normal cervical cells, 97.3% of intraepithelial neoplasia II–III, and 100% of squamous cell carcinomas. This result confirms the previous studies wherein HPV infection was found to be associated with an increased risk of cervical disease severity, since the rate of infection increased from normal cervical cytology to minor cervical intraepithelial neoplasia II–III and to squamous cell carcinoma. However, the prevalence of HPV DNA in previous reports was lower than that found in our study; this might be as a result of the different types of specimen, primers, and techniques.

A meta-analysis of HPV types in invasive cervical cancer worldwide indicated that the adjusted HPV prevalence was significantly higher in studies using both cervical exfoliated cells and biopsies (92.5%) than that determined using either cervical exfoliated cells (78.9%) or fixed biopsies (83.3%).[20]

For PCR primers, the highest HPV prevalence was obtained in studies using SPF10 primers (97.2%), whereas the lowest was obtained in studies using only type-specific PCR (74.7%). The adjusted overall HPV prevalence varied between 77.8% and 90.1% for other primer sets, but these differences were not statistically significant.[20] For the techniques of HPV DNA detection, there are many reports of HPV DNA detection in Thai women. A study that used hybrid capture II (HC-II) for HPV DNA detection reported that HR HPV was detected in all cases (100%) of high-grade dysplasia and squamous cell carcinomas, but in only 27.3% of the cases with low-grade dysplasia and 3.4% of those with a normal cytology.[5]

A population-based study using single PCR using primers GP5+/GP6+ found that the HPV DNA prevalence in cervical cells from women in Lampang and Songkla, Thailand was 4.8% in normal cervical cells, 61.5% in low-grade squamous intraepithelial lesions, and 100% in high-grade squamous intraepithelial lesions/squamous cell carcinomas. The HR HPV was found in 2.9% of normal cytology, 53.8% of low-grade squamous intraepithelial lesions, and 100% of high-grade squamous intraepithelial lesions/squamous cell carcinomas.[6]

The nested PCR using primers GP5+/GP6+ presented a higher sensitivity for HPV DNA detection than the single PCR. Remmerbach et al. performed a nested PCR of GP5+/GP6+ primer systems, which increased the positivity of HPV DNA detection from 35.8% to 65.1% in oral samples. For cervical samples, HPV DNA was increased from 46.4% to 69.6%. These studies support the higher rate of HPV DNA detection in our study.[21]

In previous studies, HPV16 was the most common type found in cytologically normal and in invasive cervical cancer worldwide.[22] A prevalence of 19.7% of HPV16 was reported in 15 613 women without cervical abnormalities in an International Agency for Research on Cancer (IARC) HPV prevalence survey. In Asia, HPV16 was present in 18.7% of HPV-positive women, but in 12.3% in sub-Saharan Africa, 21.4% in South America, and 25.5% in Europe.[22] In our study, 16.7% was detected in normal cervical cells, representative of women in Northeast Thailand. Our study suggests that these women are the risk group. A previous study from our group reported that women infected with HR HPV and HPV16 had an increased risk of cervical carcinoma with an odds ratio of 130.6 (95% CI 11.7–1457.0) and 12.3 (95% CI 4.3–36.4), respectively.[4] Our study also found the prevalence of HPV16 presenting in cervical intraepithelial neoplasia II–III (38.9%) and squamous cell carcinoma (75%) corresponding to results of previous studies in which 50–80% of HPV16 was reported in squamous cell carcinoma.[2,17,23–25]

Recent reports have indicated a greater risk associated with certain variants of HPV16 compared with others. It

has been proposed that variants of HPV16 may differ in incidence and outcome of infection. In our present study, the E6 open reading frame of HPV16 variants was determined using three primer sets; however, several regions of the E6 gene could not be amplified. It might be that the DNA is damaged during processing of the paraffin-embedded samples. Since E6 at nt 46—256 position was the most common variation of HPV16 in the Asian population and was commonly amplified, it was used for variant classification. By the modified phylogenetic classification, detected HPV16s were classified as E lineage (41.3%) and As lineage (58.7%). This supports the hypothesis that HPV16 variants have a significant geographic diversity.

The As lineage was the most common variant found, corresponding with a previous report in the Southeast Asian population.[11,15,23] Vaeteewoottacharn et al. characterized HPV16 DNA variants in the E6 and E7 coding regions of Thai cervical squamous cell carcinomas. A mutation T178G, change from aspartate to glutamate (D25E), was the most common variation accounting for 70%.[26] Choi et al. investigated the genomic variation of HPV16 E6 and E7 genes from cervical swabs of Korean commercial sex worker by PCR. They found that the dominant HPV16 E6 and E7 variants were E6 D25E 68% and E7 N29S 73%, which belong to the As lineage. They suggest that the distinctive distribution of HPV16 As variants E6 D25E and E7 N29S might be associated with geographical dependence rather than disease progression.[27]

The E lineage was commonly found in normal cervical cells (77.8%) as shown in Table 4, whereas the As lineage was found at an increasing rate with increasing histologic grade, i.e., 22.2%, 57.1%, and 73.9% in normal cervical cells, cervical intraepithelial neoplasia II—III, and squamous cell carcinomas, respectively. This result shows the associated risk of women infected with the As lineage (OR 7.292; 95% CI 1.311—40.542) compared with the E lineage.

Xi et al. reported that infection with the non-European variants is associated with a 2- to 9-fold increased risk of cervical cancer and precancerous lesions.[28] Hildesheim and Wang determined that there was an association between natural variants of HPV16 and the risk of biopsy-confirmed cervical intraepithelial neoplasia II or III. The prototype-like and non-prototype-like variants were determined by means of single-strand conformation polymorphism (SSCP). They suggested that the risk of developing cervical intraepithelial neoplasia II—III is not the same with all variants of HPV16 and that the non-prototype-like variant conferred a greater risk than the prototype-like variants.[29] Grodzki et al. suggested that the HPV16 E6 variants were significant risk factors for viral persistence and progression to a high-grade lesion.[30]

In conclusion, this study found that the prevalence of HPV and HPV16 infection is associated with an increased risk for cervical cancer and its precursor lesions, such as cervical intraepithelial neoplasia II—III. The As lineage of HPV16 variants and nucleotide variations at E6 may involve a change in the biological properties and a change in the transforming potential, increasing the risk of cervical cancer development. HPV16 As variants associated with disease severity in the population of Northeast Thailand may be important for designing a vaccine for this region.

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